REMARKS

Reexamination and reconsideration of the subject application are respectfully requested in light of the following remarks.

Status of the Claims

Claims 1-12 and 14-18 are pending. Claims 7 and 18 have been withdrawn from consideration. Claims 1-6, 8-12, and 14-17 stand rejected.

Rejections Under 35 U.S.C. § 103(a) - Harigai and Mayer

Claims 1-6, 8-12, and 14-17 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Harigai et al., "Preferential Binding of Polyethylene Glycol-Coated Liposomes Containing a Novel Cationic Lipid, TRX-20, to Human Subendothelial Cells via Chondroitin Sulfate," 18(9) Pharmaceutical Research 1284-1290 (September 2001) ("Harigai") in view of U.S. Patent No. 5,616,341 to Mayer et al. ("Mayer"). The rejection is respectfully traversed.

The Invention

Claim 1 is directed to a liposome preparation comprising a unilamellar vesicle having an interior aqueous phase at a pH 5 or less and a drug loaded therein. The unilamellar vesicle is modified with a hydrophilic macromolecule, but only on its exterior surface. The hydrophilic macromolecule is introduced as a phospholipid derivative of the hydrophilic macromolecule.

Claims 2-6, 8-12, and 14-17 depend from claim 1, incorporating all the features of claim 1 therein and reciting additional features which may be found in particular embodiments thereof.

Content of the Prior Art

Mayer describes liposomes made by the method that was conventional at the time and which remains the conventional method of liposome preparation. In the method exemplified

by Mayer, liposome forming lipids are dissolved in a volatile organic solvent and then dried into a thin film. The film is then hydrated with an aqueous buffer solution. The hydrated lipids are processed through a series of freeze-thaw steps and extruded though a membrane having pores of a defined size to produce a uniform population of liposomes of the desired size. *See, e.g.,* Mayer at col. 19, Example 3.

None of the examples in Mayer demonstrates the production of liposomes comprising lipids modified with a hydrophilic macromolecule such as PEG. However, Mayer does provide an example of the conventional method of making liposomes containing more than one lipid component. *See, e.g.,* Mayer at col. 20, Example 6. In this example, egg phosphatidylcholine (EPC) and cholesterol were mixed in chloroform, dried into a thin film, hydrated in aqueous solution, and then processed by freeze-thaw and extrusion through a membrane.

An example of how the conventional method of preparing liposomes was used to prepare liposomes modified with PEG can be found in U.S. Patent Number 5,213,804 ("Martin"). *See, e.g.,* Martin at cols. 32-33, Example 10. Here, again, vesicle-forming lipids including a PEG modified lipid were dissolved in chloroform, dried to a thin lipid film, hydrated with an aqueous solution, and processed through freeze-thaw cycles and extrusion through a membrane. Another example may be found in a paper by Lu et al., 95 Journal of Pharmacological Sciences 381-89 (2004) at 382. Lu et al. reported the preparation of pegylated doxorubicin liposomes using essentially the same method as Mayer (using sonication instead of freeze-thaw).

Taking the state of the art as a whole, it is apparent that the method of making liposomes that is exemplified by Mayer and Martin and Lu et al. was, and indeed still is, the conventional method of making liposome's used in the art. This method inherently produces liposomes that are distinct from those that are presently claimed.

Differences Between The Prior Art And The Invention

In contrast to the claimed invention, the conventional method of producing vesicles or liposomes results in a more-or-less uniform distribution of lipid components on the interior and exterior faces of the lipid bilayer. This is because the lipid components of the liposome are uniformly mixed together by being dissolved in an organic solvent at the start of the process. *See, e.g.,* Stealth Liposomes: Local Chemotherapy, BIOimaging, 2008, at 2, Illustration of a Stealth Liposome, *retrieved from* http://www.bioimaging.dk/index.php?id=75 (attached) (*It should be noted that this illustration of a doxorubicin containing pegylated liposome having PEG on both the interior and exterior surfaces was published in 2008, evidencing the continuing conventional nature of liposomes having a uniform distribution of components on both interior and exterior surfaces.*)

The conventional methods of Mayer and Martin and Lu et al. will not result in a liposome that is modified with a hydrophilic macromolecule only on its exterior surface as recited in the claims.

Harigai reported a study to determine whether the cationic lipid TRX-20 provided preferential binding of PEG-coated liposomes to targeted cells. Among the experiments reported by Harigai, one experiment varied the amount of PEG-modified lipid in the liposome and reported that the degree of preferential binding was affected by the amount of PEG. The method that Harigai used to prepare the vesicles is a method by which a hydrophilic macromolecule may appear only on the exterior surface. However, there is no explanation in Harigai of their reason to make vesicles in this manner. Certainly, there is no reason suggested for making the vesicles in this manner that would apply to the manufacture of liposomes having an interior phase at a pH < 5 for delivery of a drug that is unstable at higher pH. The vesicles described by Harigai had an interior phase at neutral pH and were not loaded with any drug.

There is no Rational Reason to Combine the References

The Examiner has contended that it would have been obvious to make the liposomes of Haragai with an acidic interior and load them with a drug as described by Mayer. Applicants respectfully point out that an invention composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. *KSR Int'l Co. v. Teleflex Inc.*, 550 US 398, 418, 82 U.S.P.Q.2d 1385, 1388 (2007). To make out a prima facie case requires a rational supported by sound scientific reasoning for modifying the prior art to arrive at the claimed combination arranged as required by the claim. *Id.*

The Office has not identified any reason for one of ordinary skill in the art to deviate from the conventional method exemplified by Mayer, which is simple and reliable. The conventional method requires fewer steps than the method of Haragai. The method of Harigai first requires one to prepare liposomes without PEG-modified lipids in the conventional manner, and then in a separate additional step the PEG-modified lipid is introduced.

This is why, in the attached Declaration, Masashi ISOZAKI testifies that prior to the discovery that led to the applicants' invention, it would not have been obvious to deviate from the conventional method of Mayer by using the method of Harigai. *See* Declaration at ¶¶ 10-18. Adding more steps to a process inherently increases the cost of a process in terms of time, effort, materials, risk of error, and efficiency. *See* Declaration at ¶ 18.

According to the testimony of Masashi ISOZAKI, there are several ways of introducing a modification only to the outside surface of a liposome. However, introducing a modification only to the outside surface of a liposome always requires performing additional steps beyond the conventional method of liposome preparation, regardless of how the modification is introduced. Consequently, no matter how it was to be done, it would not be

obvious to make a liposome having a modification that is only on the outside surface unless there was some specific reason to make it that way. *See* Declaration at ¶ 19. No such reason can be found in Mayer and/or Harigai, or generally in the knowledge in art as a whole at the time that the invention was made.

In seeking to optimize any method, one will generally seek to reduce the number of steps, the time, the effort, the amount of materials, and the risk of error. Thus, it would have been against common sense for one to endeavor to make liposomes having a specialized structure which required one to perform additional steps and take additional time to produce. It would not be obvious to modify the liposome structure without an expectation that the product would have advantages that were of greater value than the costs imposed by the added steps required to make the modification.

Neither Harigai nor Mayer suggests any advantage associated with having PEG-modified lipids only on the outside surface. The prior art of record provides no reason to make a liposome containing an interior phase at pH < 5 comprising a drug that would be unstable at high pH that is modified to have a hydrophilic macromolecule only on the exterior surface.

The Prior Art Did not Appreciate the Nature of the Problem Solved by the Claimed Invention

Vesicles containing lipids modified with a hydrophilic macromolecule suffer from a lack of stability if the interior phase is maintained at an acidic pH. This becomes particularly problematic when the vesicles are to be used to deliver drugs that are unstable at neutral pH or higher, as described, *e.g.,* in paragraph [0007] of the application. Such drugs should be maintained in vesicles or liposomes that have an acidic interior environment. The inventors studied this problem and discovered both the nature of the problem and its solution.

The inventors discovered that the vesicles were being destabilized because lipids modified with PEG were degraded to a lyso form in the presence of buffered solutions at pH < 5. As described in the specification, and in the Declaration of Masashi ISOZAKI presented herewith, PEG_{5000}-DSPE was dissolved in 4 buffers at having a pH to a concentration of 5 mg/ml, and the solution was heated at 65° C for 90 minutes. PEG_{5000}-DSPE was also dissolved in the same buffer to a concentration of 10 mg/ml, and the solution was stored at 40° C. for 1 week. Heating the PEG_{5000}-DSPE to 65° C for 90 minutes had no observable effect in any of the tested pH buffers. Likewise, there was no observable change in the lipids stored for a week in buffers having a pH > 5.

However, the PEG_{5000}-DSPE that was stored for one week in the moderately acidic pH 4 buffer demonstrated clear degradation of PEG_{5000}-DSPE to lyso-PEG. *See* Declaration at ¶¶ 35-40. This explains the destabilization of liposomes comprising PEG-lipids having an acidic interior phase during storage.

Neither Mayer nor Harigai suggested that a buffer with a pH < 5 could cause degradation of PEG-modified lipids.

The Prior Art Did Not Anticipate the Advantages Provided by the Claimed Invention

As demonstrated by the experiments presented in the specification and described in the Declaration of Masashi ISOZAKI presented herewith, the inventors discovered that hydrolysis of the lipids that comprise a vesicle membrane that include lipids that are modified by a hydrophilic polymer could be substantially prevented if the hydrophilic polymer is only present on the outside.

To demonstrate the effect of the invention, an experiment was conducted to compare an example of the liposomes as claimed (Example 1) with an example of liposomes structured as those found in the prior art (Comparative Example 1). For Example 1, the

inventors prepared liposomes in which PEG_{5000}-DSPE was only on the exterior surface. *See* Declaration at ¶¶ 22-28. For Comparative Example 1, the inventors prepared liposomes comprising PEG_{5000}-DSPE according to the conventional methods so that the PEG_{5000}-DSPE would be on both the interior and exterior surfaces. *See* Declaration at ¶¶ 29-34.

The liposomes had an interior phase at pH 4 and were maintained in solution having an exterior pH of 7.5. After two weeks storage at 40° C a distinct reduction in the amount of PEG_{5000}-DSPE was observed in the liposome having the PEG_{5000}-DSPE on both the interior and exterior surfaces, Comparative Example 1. By contrast, the loss of PEG_{5000}-DSPE was substantially prevented in the Example 1 liposomes, which had PEG only on the outside surface. *See* Declaration at ¶¶ 41-43.

Thus, the present invention provides advantages that were not suggested or predicted by the prior art. Neither Mayer nor Harigai suggested that the problem of liposome stability for storage and delivery of drugs which must be maintained at low pH could be solved by including a hydrophilic macromolecule only on the exterior liposome surface of a unilamellar vesicle.

For at least the foregoing reasons, the rejection does not make out a prima facie case of obviousness. To make out a prima facie case of obviousness, there must be some rational basis for modifying the prior art to arrive at the invention. The prior art does not provide any such basis. The Declaration of Masashi ISOZAKI explains why it would not have been obvious to combine the references as proposed by the Office without a compelling reason, which is wholly absent from the prior art.

Moreover, the invention must be considered objectively non-obvious because, as further explained in the Declaration of Masashi ISOZAKI, the present invention has demonstrated advantages that were neither suggested nor predicted by the prior art. Withdrawal of the rejection is appropriate and is respectfully requested.

CONCLUSION

In the event that there are any questions relating to this Reply, or to the application in general, it would be appreciated if the Examiner would contact the undersigned attorney by telephone at (703) 836-6620 so that prosecution of the application may be expedited. The Patent Office is hereby authorized to charge any necessary fees, or credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,
BUCHANAN INGERSOLL & ROONEY PC

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